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FABRICATION OF 45S5 BIOGLASS® COMPOSITE SCAFFOLD

RINGKASAN: *Kejuruteraan tisu "osteochondral" adalah salah satu bidang yang sedang dikembangkan kerana ia mampu mengatasi pelbagai penyakit seperti osteoartritis. Kertas kerja ini membincangkan tentang bahan bioseramik iaitu 45S5 Bioglass® dengan komposisi 45% SiO_2, 24.5 % Na_2O, 24.5 % CaO dan 6 % P_2O_5 sebagai bahan perumah (scaffold) komposit untuk diimplan diantara tulang rawan dan tibia di bahagian lutut. Bahan di atas difabrikasi menggunakan kaedah replikasi busa yang mengandungi dua langkah utama iaitu (1) penyediaan jasad anum dan (2) pensinteran. Perumah komposit yang dihasilkan telah melalui beberapa teknik pencirian iaitu kajian morfologi melalui Variable Pressure Scanning Electron Microscope (VPSEM), pengujian tensil melalui Universal Tensile Machine (UTM) dan kajian fasa bahan melalui X-ray diffraction (XRD). Mikrograf SEM menunjukkan kehadiran struktur poros pada perumah yang mengandungi liang mikro dan nano. Spektrum XRD pula menunjukkan kehadiran fasa $Na_2Ca_2Si_3O_9$ bagi perumah yang telah tersinter. Perumah 45S5 Bioglass® yang disinter pada suhu 1000°C selama 2 jam memberikan nilai kekuatan mampatan 0.32 MPa. Ujian bioaktif di dalam simulasi cecair badan (SBF) pada 7, 14 dan 28 hari menunjukkan lapisan nipis apatit terbentuk di atas permukaan perumah.*

ABSTRACT: Recently, osteochondral tissue engineering have attracted great attention because a variety of diseases such as osteoarthritis. Expands in this tissue engineering, it has led researches to approach a new device such as a 45S5 Bioglass® composite scaffold as interfacial tissue, which will be implanted between cartilage and tibia at knee site. The composition of 45S5 Bioglass® composite are 45 % SiO_2, 24.5 % Na_2O, 24.5 % CaO and 6 % P_2O_5. This paper will describe the fabrication of 45S5 Bioglass® composite scaffolds which will be the layer for bone growth in the constructs for osteochondral tissue engineering. The fabrication of 45S5 Bioglass® scaffold was carried out using foam replication technique which consists of two steps; (1) preparation of green body and (2) sintering of 45S5 Bioglass® scaffold. Characterisations of the above scaffold were carried out using Variable Pressure Scanning Electron Microscope (VPSEM), INSTRON Universal Tensile Machine (UTM) and X-ray diffraction (XRD). The SEM micrographs revealed the porous structure of the scaffold consisting of micropores and nanopores. The X-ray diffraction (XRD)

spectrum showed the presence of $Na_2Ca_2Si_3O_9$ phases in the sintered scaffold. 45S5 Bioglass® scaffold sintered at 1000 °C for 2 hours gave the compressive strength of 0.32 MPa. Bioactivity assessment in Simulated Body Fluid (SBF) for 7, 14 and 28 days showed the formation of apatite layer on the scaffold.

Keywords: Green body, 45S5 Bioglass® scaffold, tissue engineering.

INTRODUCTION

Tissue engineering has emerged as a promising approach for the repair and regeneration of tissues and organs lost or damaged as a result of trauma, injury, disease or aging. It has the potential to overcome the problem of a shortage of living tissues and organs available for transplantation. In the most common approach, a biomaterial scaffold with a well-defined architecture serves as a temporary structure for cells and guide their proliferation and differentiation into the desired tissue or organ (Mohamed, 2011).

To fulfill the above characteristics, there are several specific criteria for ideal scaffolds used in bone tissue engineering, and was summarized as follows: ability to deliver cells, excellent osteoconductivity, good biodegradability, appropriate mechanical properties, highly porous structure with porosity of more than 90 % volume and pore size between the range of 400-500 µm, irregular shape fabrication ability and commercialization potential (Qizhi *et al.*, 2006, Antonio *et al.*, 2004).

The selection of the most appropriate material to produce a scaffold for bone tissue engineering is a very important step towards the construction of a tissue engineered product, since the properties will determine the properties of the scaffold (Gomez *et al.*, 2002). There are several potential materials that have been widely known suitable for the above applications such as ceramics, metals and polymers both from natural and synthetic origins have been developed.

The possible materials are bioactive silicate glass (e.g. 45S5 Bioglass®) with the composition in the system of SiO_2-Na_2O-CaO-P_2O_5 and were discovered by Hench in 1969 (Chen *et al.*, 2006). This potential material meets the first three of the above scaffold criteria which are excellent osteoconductivity and bioactivity (Wilson *et al.*, 1981), ability to deliver cells (Gatti *et al.*, 1994) and controlled biodegradability (Clark *et al.*, 1994). These advantages make bioactive glasses as promising scaffold materials for tissue engineering. There are several techniques in fabrication of porous scaffold such as the replication technique (Qizhi *et al.*, 2006), solvent casting, phase invasion, fiber bonding, melt based technologies, freeze drying and rapid prototyping (Antonio *et al.*, 2004). The synthetic polymers that are widely used for tissue engineering scaffolds including polyglycolic acid (PGA) and poly-

(L)-lactic acid (PLLA) or their copolymers (PLGA). PDLLA has been extensively used as biomedical coating of orthopeadic materials because it shows excellent biocompatibility in vivo and good osteoconductive potential (Seal *et al.*, 2001).

The objective of this work, therefore, was to fabricate 45S5 Bioglass® scaffold using foam replication technique to achieve mechanically stable 3-D scaffold through sintering temperature of 1000 °C and further coated with poly (D,L-lactic acid) PDLLA to increase the strength of the scaffold. The material was characterised using VPSEM, XRD and UTM. The bioactivity of the 45S5 Bioglass® -polymer composite was assessed by immersion in Simulated Body Fluid (SBF).

EXPERIMENTAL

Materials

A melt derived 45S5 Bioglass® powder (average particle size of 53 µm) was purchased from Mo-Sci, USA. The reticulated polyurethane foam (PU) with 45 ppi (pores per inch) was purchased from Recticel, Belgium. The foam was cut into size of 10 mm width x 10 mm length x 20 mm height. Polyvinyl alcohol (PVA), 99 % hydrolysed (Mw=85,000-124,000) used as a binder was purchased from Sigma-Aldrich, Malaysia. PDLLA with inherent viscosity of 2.15 dL/g used as coating material was purchased from Purac, Biochem, Netherlands. The dimethyl carbonate (DMC) used as solvent was purchased from Sigma-Aldrich, Malaysia.

Scaffold fabrication

The recipe of the slurry was prepared by dissolving 5 g of PVA in 50 ml H_2O at 80 °C, with stirring for 1 hour. 30 g of 45S5 Bioglass® was then added and stirred for at least 1 hour. The PU foam with dimension of (10 x 10 x 20) mm^3 was immersed in the Bioglass® slurry for 2 minutes and the extra slurry was squeezed out vigorously by hand. The obtained green body was then oven (Memmert ULM 500) dried at 60 °C overnight. The burning out of the PU foam was at 550 °C for 3 hours with heating rate at 2 °C/min. The sample was sintered at 1000 °C using high temperature furnace (CS5) for 2 hours with the cooling rate of 5 °C /min as shown in Figure 1.

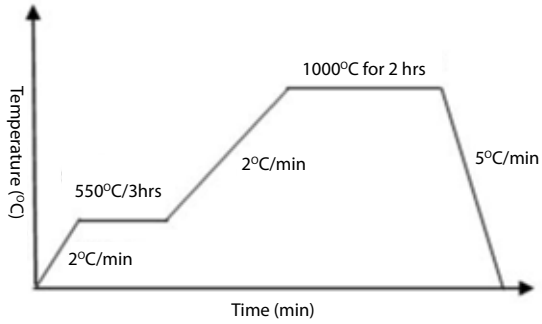


Figure 1. *Heat treatment profile for burning out the PU foam and sintering the 45S5 Bioglass® green bodies*

Coating of 45S5 Bioglass® scaffolds

The dimensions of the sintered 45S5 Bioglass® scaffolds were measured using a digital caliper (Mitutoyo, UK) and the weight of the scaffolds was measured using an electronic analytical weighing balance (Ohaus,USA). The scaffolds were slowly immersed in a solution of 5 % wt/v PDLLA-DMC for 1 hour. After 1 hour immersion time, the scaffolds were taken out from the PDLLA-DMC solution and placed on tissue paper to dry at room temperature for at least 12 hours. Finally, the weight of the dried scaffolds was measured using an electronic analytical weighing balance and the dimensions were measured again using a digital caliper (Mitutoyo, UK). For samples with triple coatings (3XC), the above procedure was repeated three times to increase the strength of the scaffolds.

Characterisations

1) **Porosity**

The porosity of the sample was measured using mass and volume of the foams by the equation below:

$$P = 1 - \frac{\rho_{foam}}{\rho_{solid}} = 1 - \rho_{relative} \qquad (1)$$

Where the density of scaffolds, ρ_{foam} was determined from the mass and dimension of the sintered scaffolds (ρ_{foam} = mass/volume) and the density of solid 45S5 Bioglass®, ρ_{solid} = 2.7 g/cm^3.

2) **Phase and microstructural analysis**

The crystallinity of the sintered samples and formation of hydroxyapatite (HA) before and after immersion in the SBF was performed using X-ray diffraction (XRD) (Bruker D8 Advanced). The data was collected over the range of 2θ =

20-80 ° using step size of 0.040 °. The microstructures of the samples before and after immersion in SBF were performed using Variable Pressure Scanning Electron Microscope (VPSEM) *(Carl Zeiss EVO LS 10)*. Energy Dispersive X-Ray (EDS) spectra (Kα) of samples after immersion in SBF were collected at 10-15 kV and were processed using INCA (Oxford Instrument) programme.

3) Mechanical testing

The compression strength of the uncoated and coated samples was determined using Universal Tensile Machine (UTM) *(INSTRON 3369)* with crosshead speed of 2 mm/min with the sample dimension of (10 x 10 x 20) mm^3. The load was applied until compression achieved 70 % of the overall dimension. At least five samples were tested to get average results (n=5).

4) SBF treatment of 45S5 Bioglass® scaffolds

SBF solution was prepared according to the standard procedure (Takamada *et al*., 2006). The 45S5 Bioglass® scaffolds coated once (C) and three times (3XC) were immersed in 30 ml SBF in a clean centrifuge tube. The 45S5 Bioglass® as-sintered scaffolds (UC)(uncoated samples) were also immersed in the SBF to compare the behaviour. The samples were then placed in an incubator at a controlled temperature of 37 °C. The size of the samples were (5 x 5 x 5) mm^3. Samples were extracted from the SBF solution after 1, 2 and 4 weeks. The SBF was replaced twice a week because the cation concentration changed during the course of the experiment and also to simulate a dynamic flow of SBF (Chen *et al.*, 2006). Once removed from the incubator, the samples were rinsed gently with deionised water and left to dry at ambient temperature.

RESULTS AND DISCUSSION

The porous 3D 45S5 Bioglass® scaffolds was produced using foam replication technique or also called polymer-sponge method. By using this method the scaffold was produced with a highly porous structure which can mimic the spongy bone or cancellous bone inside the body. The fabricated scaffold using the foam replication technique followed a sintering process as shown in Figure 1. The as-sintered sample (UC) produced was then coated with 5 % wt/v PDLLA once (C) and triple times (3XC).

The porosity of the scaffold was calculated using Equation 1. The results obtained showed that the porosity decreased from as sintered sample (UC) to sample coated three times with 5 % wt/v of PDLLA (3XC) as shown in Figure 2. The decreased in porosity was expected due to the PDLLA filled the porous structure. Although the porosity decreased (94 % - 89 %), but it still achieved nearly 90 % porosity. High porosity is valuable because it allows cell penetration, tissue in growth and vascularisation, and nutrients delivery to the bone (Paola *et al*., 2010).

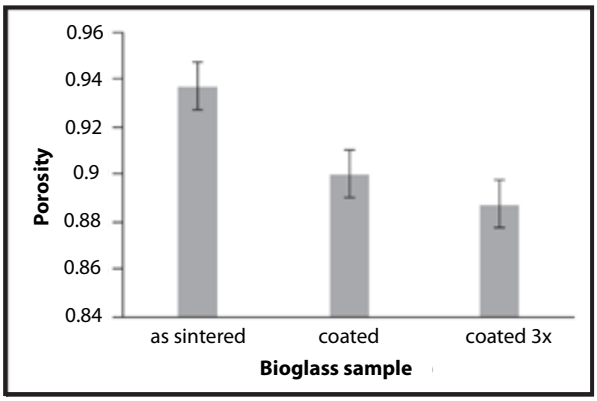
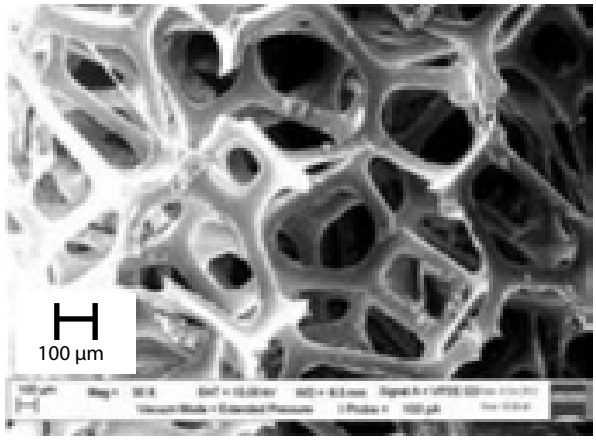


Figure 2. *Porosity of scaffold decreased after coated with PDLLA (n=5, p<0.05)*

Figure 3(a) showed the morphology of as received PU foam with pore size in the range of 500 -800 µm. While Figure 3(b) showed the morphology of 45S5 Bioglass® based scaffolds with pore size in the range of 400–700 µm after sintering process. The 45S5 Bioglass® based scaffolds pore size decreased due to the shrinkage of scaffolds after sintering at 1000 °C for 2 hours which is expected during the fabrication of foam replication technique (Qinzhi *et al.*, 2006). The morphology of highly porous 45S5 Bioglass® composite scaffolds with PDLLA were illustrated in Figures 4(b–c). Figure 4(a) showed the morphology of the 45S5 Bioglass® scaffold strut due to the densification of the bioglass occurred by a viscous flow sintering mechanism in glass (Qizhi *et al.*, 2006) after sintered at 1000 °C for 2 hours. Figures 4(b) and (c) revealed the strut's morphology of the 45S5 Bioglass® scaffolds after coating with PDLLA for once (C) and triple times (3xC) respectively. It was clearly shown on the micrograph that the polymer coating was thicker in Figure 4(c) than in Figure 4(b).

Figures 4 (a-c) shows that the struts were partially densified where the bioglass powder is not fully melted to form a smooth surface after sintering at 1000 °C for 2 hours, thus leaving micropores on its surface. It is also shown that the scaffolds produce large macropores in the middle of the strut which is the characteristic of fabrication using sponge replication method (Qinzhi *et al.,* 2006). The results also indicated that this method produced a highly porous scaffold which can enhanced the cell proliferation as well as high pore distribution and interconnectivity as shown in Figure 3 (b).



(a)

(b)

Figure 3. *Morphology of (a) PU foam and (b) 45S5 Bioglass® based scaffolds*

(b)

(c)

Figure 4. *SEM struts microstructure of (a) as sintered (UC), (b) coated with 5 wt% PDLLA(C) and (C) 3x coating with 5 wt% PDLLA (3xC)*

The compressive strength of the scaffold was determined using universal testing machine as shown in Figure 5. The compressive strength increased from 0.08 MPa to 0.32 MPa after coating three times with 5 wt/v% PDLLA (3xC) samples sintered at 1000 °C for 2 hours. The increase in the mechanical strength of the coated samples as compared to the as sintered scaffolds (UC) is due to the PDLLA polymer infiltration into the micropores of the partially sintered scaffolds. During the coating process, the polymer will infiltrate into micropores and microcracks of the struts and toughen the struts by reducing the shearing effect. This will increase the toughness of the scaffolds as shown by previous studies (Clupper *et al.*, 2001, Yunos *et al.*, 2008). It is also shown that compressive strength of scaffolds coated three times (3xC) is slightly higher than scaffolds coated once (C) which can be contributed by the thicker layer of polymer coatings which will increase the mechanical stability and the toughness of the struts.

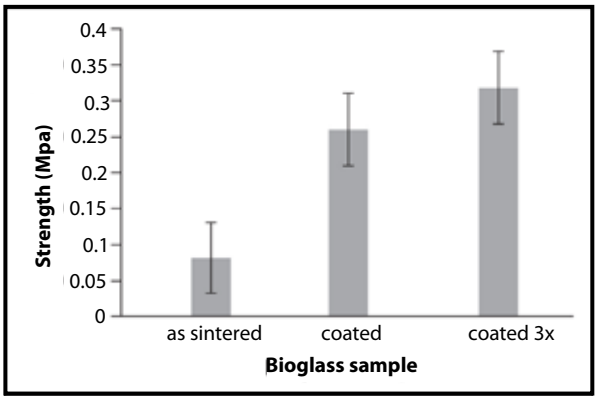


Figure 5. *Compressive strength of 45S5 Bioglass® scaffold sintered at 1000 °C for 2 hours and after coated with PDLLA (n=5, p<0.05)*

The XRD micrographs of 45S5 Bioglass® are revealed in Figure 6. At room temperature, the 45S5 Bioglass® is in amorphous phase (Figure 6(a)) and it crystallized after sintered at 1000 °C for 2 hours (Figure 6(b)). The crystalline phase showed was $Na_2Ca_2Si_3O_9$ (ICCD = 22.1455) Qinzhi *et al.*, 2006. This crystalline phase is the same as previous studies on sintered bioactive glasses (Clupper *et al.*, 2001).

The concentration of naphthalene after encapsulation with a series of hyperbranched polymer of different molecular weights and concentrations are shown in Figure 3. From the bar graph, it was clearly shown that the solubility of naphthalene was zero (after free solubility of naphthalene was taken into account) and increased after encapsulation with hyperbranched polymers.

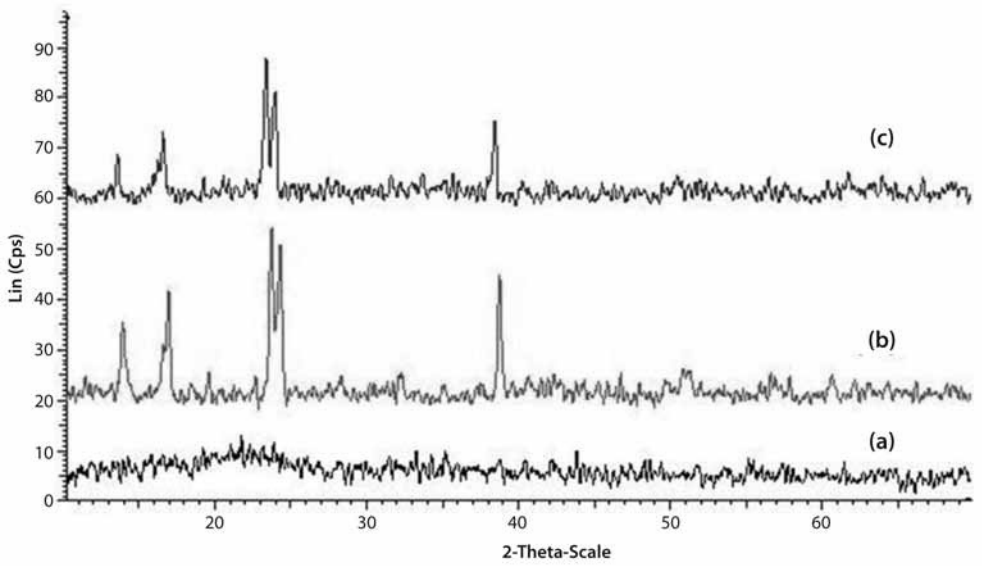
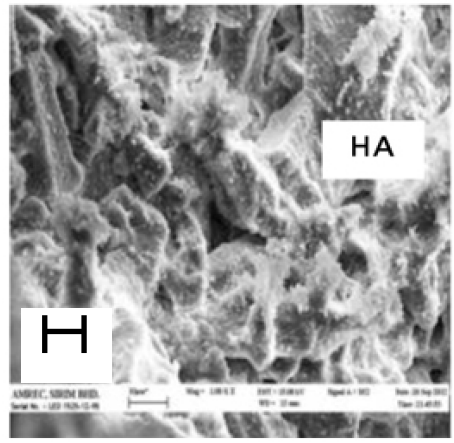


Figure 6. *XRD result of (a) 45S5 Bioglass® powder, (b) as-sintered scaffold (UC) and (c) sintered and coated once (C)*

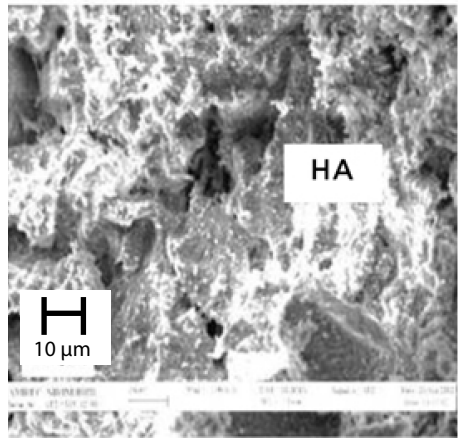
Bioactivity assessment of the 45S5 Bioglass® scaffolds

45S5 Bioglass® scaffolds sintered at 1000 °C for 2 hours (UC), scaffolds coated once (C) and triple times (3XC) with 5 % wt/v PDLLA were immersed in 30 ml solution of SBF for 7, 14 and 28 days in an incubator at 37 °C. The bioactivity assessment of the as-sintered and coated scaffolds after immersion in SBF was characterised by SEM, EDS and XRD.

Figures 7, 8 and 9 show SEM images of as-sintered scaffolds (UC), scaffolds coated once (C) and three times (3XC) with 5 % wt/v PDLLA after immersion in SBF for 7,14 and 28 days. There was a clear formation of HA phases confirmed using XRD on the surface of the scaffolds as shown in Figure 11 after 7 days immersion in SBF. This was indicated by the sparsely covered surface by apatite precipitates as shown by Figures 7(a), (b) and (c) for UC, C and 3XC samples respectively. After 14 days immersion in SBF, the surface of struts was fully covered by large amount of apatite sphere fused together as shown by Figures 8 (a), (b) and (c). After 28 days immersion in SBF, almost the whole scaffolds were covered with thick apatite layer and HA crystals as shown by Figures 9(a), (b) and (c). Previous study (Paola *et al.*, 2010) showed that HA formed when the thickness of the PDLLA coating was not homogenous due to the roughness of the original sintered struts, thus becomes perforated upon soaking in SBF. With increasing immersion time, a large surface area of the bioactive glass becomes exposed to SBF and thus HA-like crystals formed directly on the surface of the struts. Eventually the bioactive substrate was covered by a continuous layer of HA, on which agglomerates of new HA crystal were formed.



(a)



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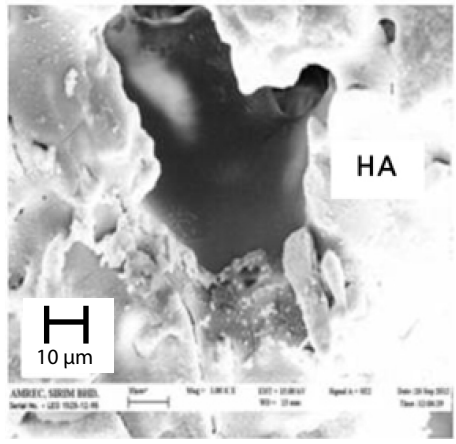
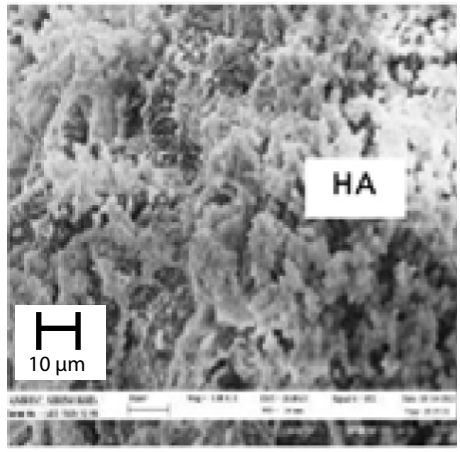
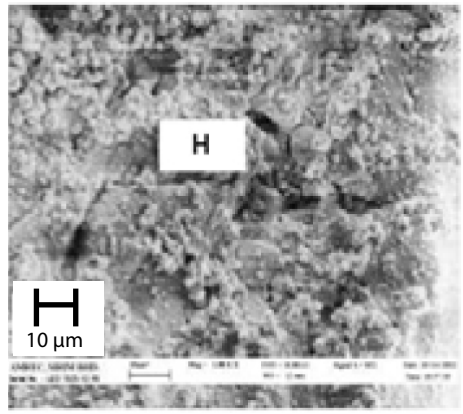


(c)

Figure 7. *Hydroxyapatite formed on the surfaces of struts after immersion in simulated body fluid (SBF) for 7 days for (a) UC, (b) C and (c) 3XC*



(a)



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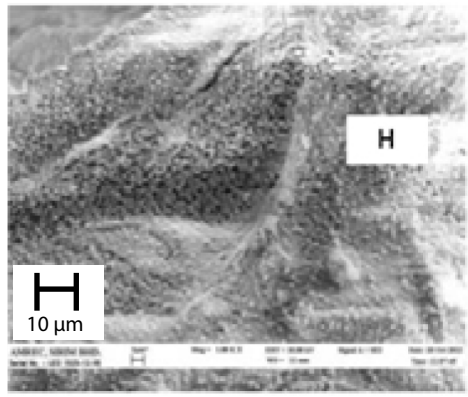
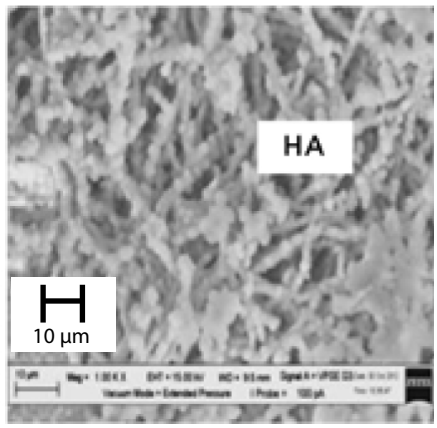
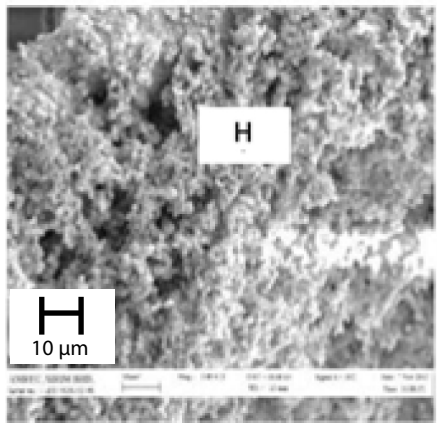


(c)

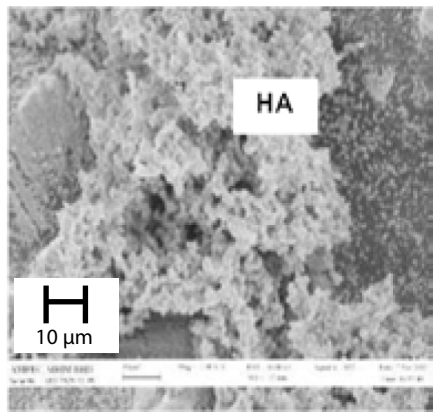
Figure 8. *Hydroxyapatite formed on the surfaces of struts after immersion in simulated body fluid (SBF) for 14 days for (a) UC, (b) C and (c) 3XC*



(a)



(b)



(c)

Figure 9. *Hyroxyapatite formed on the surfaces of struts after immersion in simulated body fluid (SBF) for 28 days for (a) UC, (b) C and (c) 3XC*

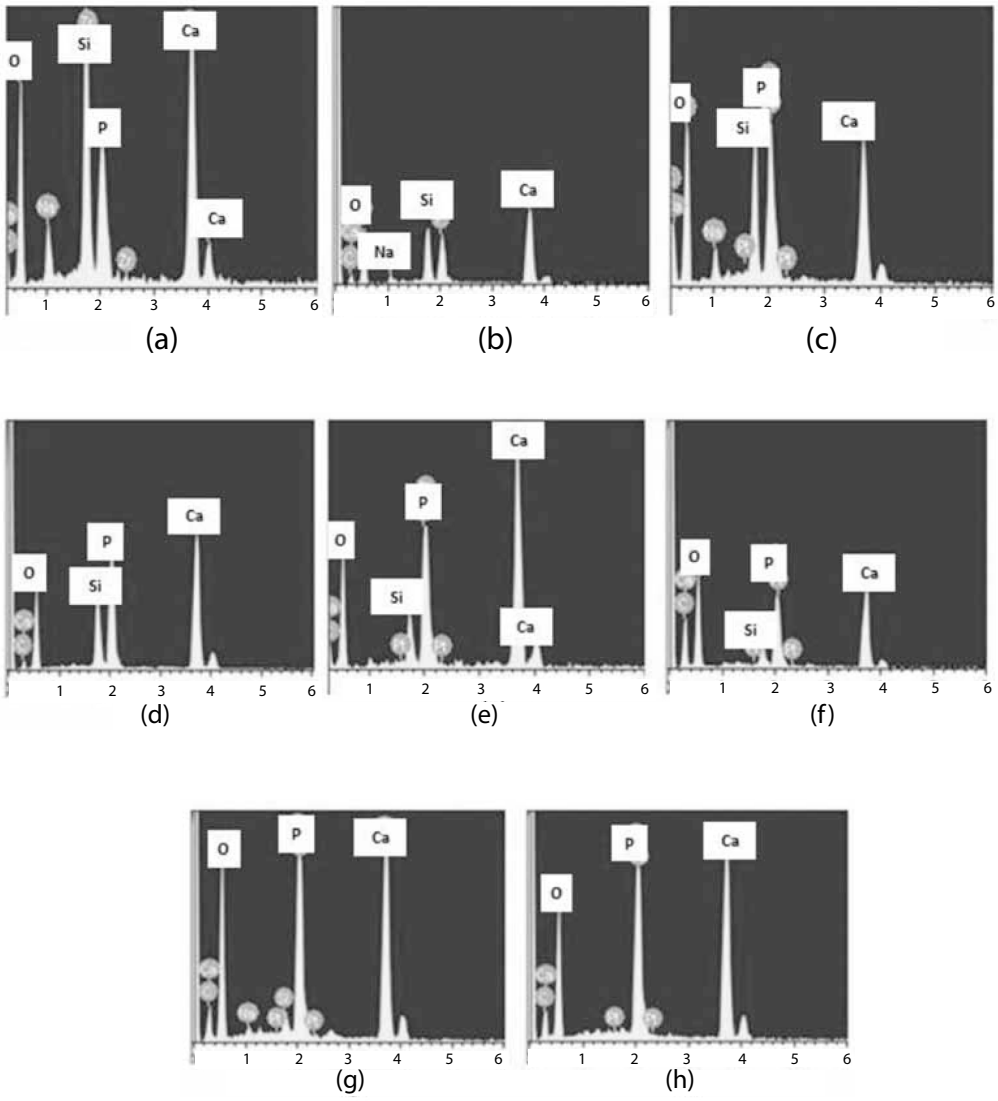


Figure 10. *EDS spectra for UC, C and 3X C samples after immersion in SBF for (a-c) 7 days, (d-f) 14 days and (g-h) 28 days respectively (Ca - 3.8 & 4.1 KeV; P- 2 KeV; Si- 1.8 KeV; Na -1 KeV; O - 0.3KeV)*

The results of SEM were confirmed by EDS analysis as shown by Figure 10. The relative intensity of the Si, Ca and P significantly changes with incubation time for UC, C and 3X C samples. It can be seen that after soaking in SBF for 14 days, the concentration of calcium (Ca) and phosphorous (P) increases, accompanied by a decrease in the concentration of Si, which strengthen the indication of extended development of apatite. After 28 days immersion time in SBF, HA layer are formed on the surface of the 45S5 Bioglass® scaffolds because the Si peaks are very small or disappeared while the calculated ratio of Ca/P is about 1.62 for samples 3XC and ratio of Ca/P is 1.53 for samples C respectively, which are comparable to ratio of Ca/P of human bone ranges between 1.5-1.7 depending on the human age (Jones *et al.*, 2001).

Assessment of bioactivity was also carried out with XRD analysis of the 45S5 Bioglass® scaffolds after immersion in SBF for 7-28 days. Figure 11 show XRD spectra of the 45S5 Bioglass® scaffolds of UC, C and 3XC after immersion in SBF for 7, 14 and 28 days. A significant pattern obtained from the XRD spectra was that the crystallinity of the sintered 45S5 Bioglass® scaffolds corresponding to $Na_2Ca_2Si_3O_9$ phase at $2(\theta) = 34°$ and 35° at 7 days were identified and decreased with increasing immersion time in SBF as shown in Figures 11 (a), (b) and (c). The sharp diffraction peaks of $Na_2Ca_2Si_3O_9$ disappeared from the XRD spectra after immersion in SBF for 28 days, leaving a typical broad halo (produced by an amorphous phase) overlapped by the sharp diffraction peaks of the HA phase (Chen *et al.,* 2006). The results of the XRD show that the coating of the 45S5 Bioglass® scaffolds do not impede the bioactivity of the 45S5 Bioglass® scaffolds as shown by previous studies (Chen *et al.,* 2006). Comparison of Figure 11(b) and (c) shows qualitatively that the apatite formation for C is slightly slower than 3XC since the Bioglass® phases are still present at 14 days for samples C which could be due to PDLLA coatings producing carboxylate anions (COO^-) for binding with calcium ions (Ca^{2+}) which can stimulates surface nucleation.

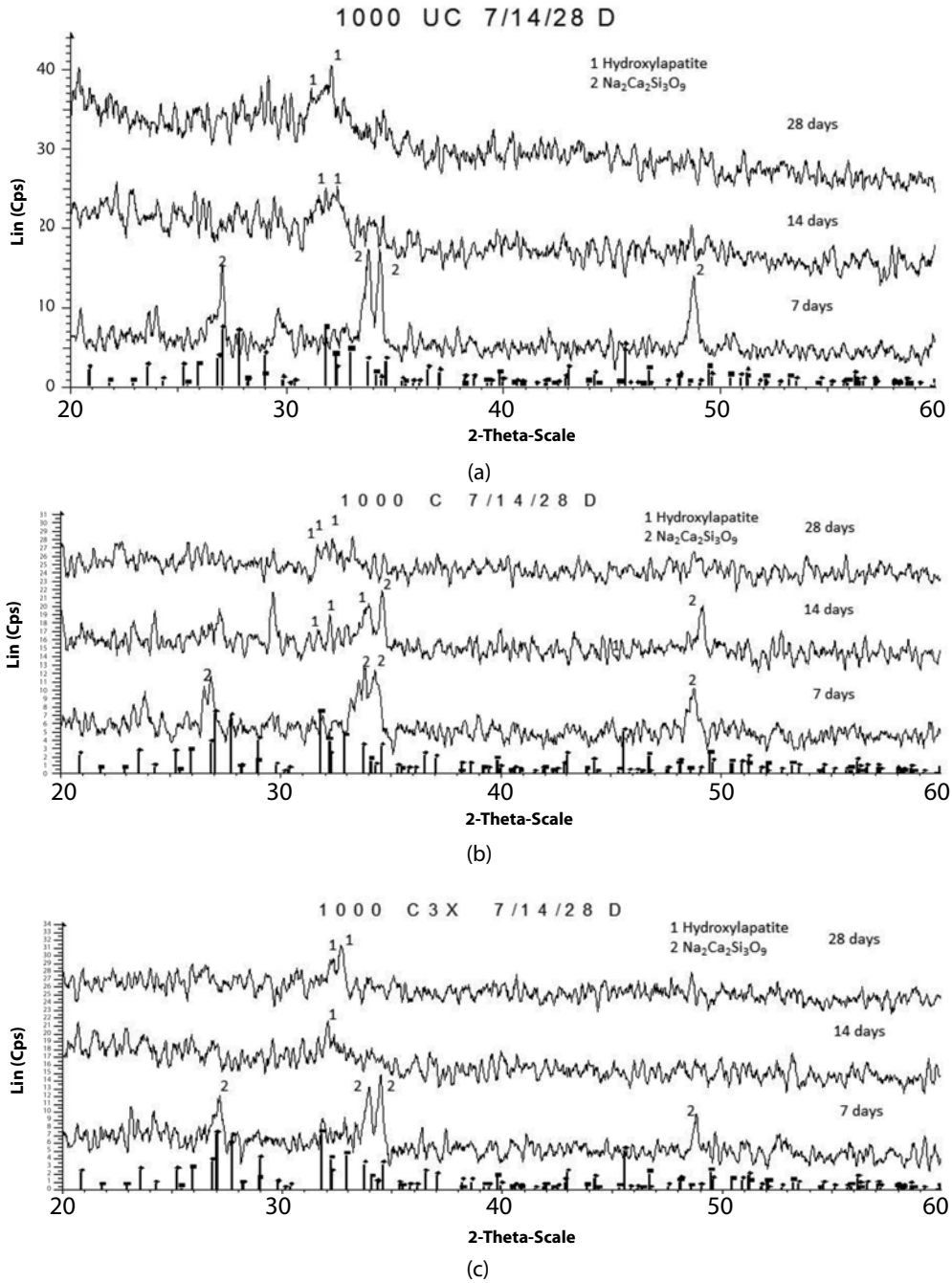


Figure 11. *XRD spectra of a) UC, b) C and c) 3X C immersed in SBF for 7,14 dan 28 weeks stacked together*

CONCLUSION

The sponge replication method used has produced highly porous 3D 45S5 Bioglass® scaffolds with interconnected pores of size range between 400-700 μm to facilitate cell growth and proliferation within the construct for potential bone tissue engineering applications. Compressive strength of the coated 45S5 Bioglass® scaffolds also increased with increased coating layers of PDLLA polymer. Bioactivity studies as revealed by SEM, EDS and XRD results show that the coatings of 45S5 Bioglass® scaffolds with 1x and 3x PDLLA will not retard the formation of HA after immersion in SBF for 4 weeks. XRD analysis also revealed that coating with 5 % wt/v PDLLA triple times (3XC) shows better HA formations than one time (C) coating after immersion in SBF qualitatively.

ACKNOWLEDGEMENT

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